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The Influence of Syringomycin on Ribonucleic Acid Synthesis[1]

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Abstract. **Syringomycin, a wide-spectrum antibiotic produced by strains of *Pseudomonas syringae* which cause bacterial canker of peach, was able to bind to salmon sperm and calf thymus deoxyribonucleic acid but not to calf thymus histone; it also inhibited ribonucleic acid polymerase activity. These abilities to bind to deoxyribonucleic acid and to inhibit ribonucleic acid polymerase were inactivated when the phytotoxic and antibiotic properties of syringomycin were inactivated.**

Syringomycin, a phytotoxin produced by *Pseudomonas syringae* van Hall that causes the bacterial canker disease of peach *Prunus persica* (L.) Batsch, is also a wide-spectrum antibiotic (1). Its general biocidal activity against cells of higher plants, fungi, bacteria, and other microorganisms apparently involves either induction of dysfunction of a metabolic process common to all these organisms, or inhibition of several vital processes one or more of which are present in all affected cells. It is even bactericidal to the strains of *P. syringae* that produce it (1). Syringomycin is a water-soluble and dialyzable polypeptide (8).

The antibiotic actinomycin D, also a polypeptide, has been shown (4) to form a complex with deoxyribonucleic acid (DNA). The formation of this complex inhibited separation of the 2 DNA strands that is otherwise induced by elevated temperature or decreased pH (7). Reich (7) showed that actinomycin D raised the transition temperature (T_m) when he followed the hypochromicity curve of calf thymus DNA. Jurkowitz (3) monitored a change in light absorbance of actinomycin D upon the addition of calf thymus RNA. The binding of actinomycin D to DNA has been shown to inhibit DNA-dependent ribonucleic acid (RNA) synthesis by RNA polymerase preferentially to the inhibition of DNA polymerase (2).

Alpha-amanitin, a polypeptide toxin isolated from poisonous mushrooms of the genus *Amanita,* has also been found to inhibit the RNA polymerase reaction at relatively low concentrations (10^{-7} M) (9).

This study was made to determine (a) whether syringomycin would readily form a complex with DNA and whether histone might alter the formation of this complex; (b) whether syringomycin would inhibit RNA polymerase action; and (c) whether any inhibition could be related to the antibiotic action of syringomycin.

Materials and Methods

Preparation of Syringomycin. The method of DeVay *et al.* (1) was used for the production of syringomycin by an isolate of *P. syringae,* B-3, which is pathogenic on peach. Concentrations of the antibiotic were purified on columns of carboxymethyl cellulose eluted with a gradient of HCl by the method of Sinden (8). Titer was determined by spotting 10 μl droplets of dilutions of the syringomycin preparations on potato-dextrose agar (PDA). The agar was then sprayed with a suspension of *Geotrichum candidum* Link ex Fries. One unit of syringomycin was defined as equal to the most dilute droplet which inhibited *G. candidum.* The purified preparation of syringomycin obtained had an activity of 1 unit per μg of residue.

Binding of Syringomycin to DNA. Calf thymus DNA (Sigma) or salmon sperm DNA (CalBiochem) was allowed to dissolve overnight in 0.01 M tris-HCl buffer (pH 7.4) containing 0.01 M NaCl. The concentration of DNA used in the binding experiment was 2.5 μg DNA phosphorus per ml. In the experiment involving the binding of calf thymus histone (Sigma) to calf thymus DNA, the ratio of histone to DNA was 1.1 mg protein per 1.0 mg DNA, determined by the method of Jurkowitz (3). All optical density measurements were made at pH 7.4 at the temperatures indicated in Fig. 1 and 2.

Ribonucleic Acid Polymerase Assay. The assay measured the incorporation of ^{14}C-labeled adenosine triphosphate (ATP) into an acid-insoluble product by an RNA polymerase prepared from *Micrococcus lysodeikticus* FLEM. by methods of Nakamoto *et al.* (6). Calf thymus DNA served as the template, and experiments were done with and without 40 μg/ml

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syringomycin in the incubation mixture. The incubation mixture, adapted from Nakamoto *et al.* (6), contained 50 μmoles tris-HCl buffer (pH 7.5 or 7.0), 1.25 μmoles $MnCl_2 \cdot 4H_2O$, 1.0 μmole $MgCl_2 \cdot 6H_2O$, 6.0 μmoles β-mercaptoethanol, 200 μg calf thymus DNA per ml, and 0.2 μmole each of CTP, GTP, UTP, and ATP. ATP was labeled as ATP-8-^{14}C, with a specific radioactivity of 1.5 μc/μmole. Syringomycin, if present, was added to a concentration of 40 μg/ml. The 10-min incubation period at 37° was initiated by adding 2 units of RNA polymerase (1.68 μg protein), giving a final incubation mixture volume of 0.5 ml. The reaction was stopped by adding 0.10 ml cold 50 % trichloroacetic acid. After 3 min at 0°, 2.0 ml of cold 5 % trichloroacetic acid were added. The material was centrifuged for 5 min at 15,000*g*, and the supernatant fluid was discarded. The sediment residue was washed twice with 2.0 ml of 5 % TCA and suspended in 0.5 ml of 2 N ammonium hydroxide, and the suspension was placed in a scintillation solution prepared by the procedure of Lui *et al.* (5).

Data shown in the figures are the average of 2 or more experiments.

Results

Syringomycin at 40 μg/ml caused a 7.0° shift in the T_m of salmon sperm DNA and calf thymus DNA (Fig. 1). The concentrations of syringomycin required to shift the melting curve are shown in Fig. 2. The shift was maximum at concentrations of 40 ppm or greater.

In the presence of calf thymus histone the melting curve of the calf thymus DNA was shifted to a higher temperature. The curve was not altered by the additional presence of syringomycin with the DNA and histone.

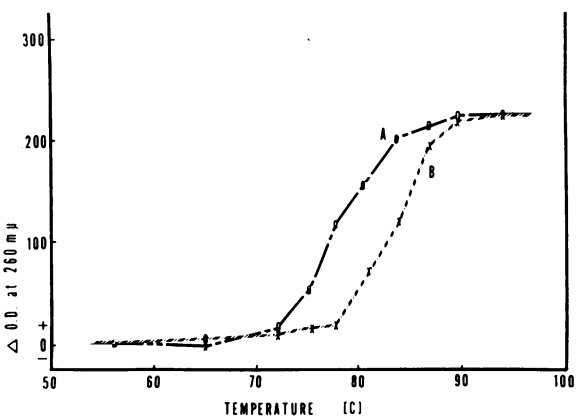

FIG. 1. Salmon sperm or calf thymus DNA (curve A) salmon sperm or calf thymus DNA in the presence of 40 μg/ml syringomycin (curve B). The reaction systems contained 2.5 μg DNA phosphorus/ml and, if syringomycin was present, 40 μg/ml syringomycin and 0.01 M tris-HCl buffer (pH 7.4) containing 0.01 M NaCl. In the presence of syringomycin in the T_m or half melting point shifted from 77.5° to 84.5°.

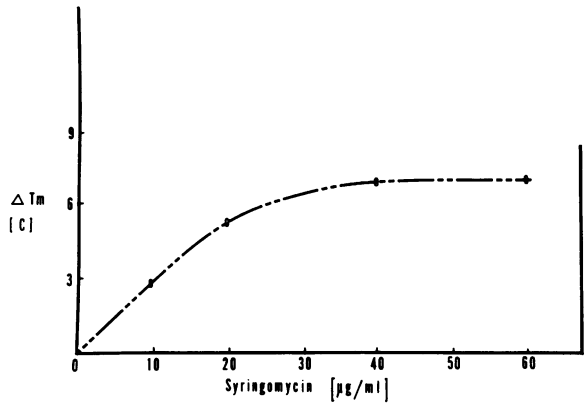

FIG. 2. The shift in the salmon sperm DNA T_m with increasing amount of syringomycin present in the reaction mixture. The conditions of the experiment were the same as those given for Fig. 1.

The percent inhibition of RNA polymerase from *M. lysodeikticus* by syringomycin with calf thymus DNA serving as the template, was 70 % at pH 7.0, 22 % at pH 7.4. The RNA polymerase activity at pH 7.0 was 60 % of that at pH 7.4. If the syringomycin was adjusted to pH 11.0 at 30° for 15 min, then readjusted to pH 7.4 for the RNA polymerase assay, no inhibition of RNA polymerase was observed. Adjustment of solutions containing 1200 units per ml of syringomycin to pH 2.0, 7.0, and 7.4 caused no reduction in their antibiotic activity against *G. candidum* for at least 1 hr after adjustment. At pH 11.0, however, total antibiotic activity was lost within 15 min. The isoelectric point of syringomycin is pH 6.9 to 7.0 (8) and during the adjustment of the solutions to pH 7.0 and 7.4, syringomycin precipitated; the titer of the supernatant solution remained at approximately 100 units per ml. When the precipitate was redissolved by lowering the pH, none of the antibiotic activity had been lost. The absorbance peak of syringomycin at 260 mμ increased approximately 13 % as the pH of the syringomycin solution (100 units) increased from pH 2.0 to 7.4. Since the solutions of syringomycin used in experiments on the melting characteristics of DNA contained approximately 40 units of syringomycin per ml, they were not affected by the precipitation of syringomycin that occurs in concentrated solutions at pH 7.0 and 7.4. Thus the decreased inhibition of RNA polymerase by syringomycin with an increase in pH from 7.0 to 7.4 was not caused by the precipitation of syringomycin.

Discussion

Syringomycin caused an upward shift in the T_m of both salmon sperm DNA and calf thymus DNA, which is indicative of the ability of syringomycin to

inhibit the separation of DNA strands induced by elevated temperatures. The amount of syringomycin required to cause this inhibition, as shown in Fig. 2, is similar to the amount of actinomycin D required to produce the same effect (3). The binding of calf thymus histone to calf thymus DNA appeared to be similar with and without syringomycin present. The syringomycin either did not bind to the histone or, if it did, had no effect on the ability of histone to form a complex with DNA.

Formation of the DNA-syringomycin complex severely inhibited RNA polymerase activity at pH 7.0. Increasing pH slightly increased the UV absorbance of syringomycin, possibly indicating an alternation in the tertiary structure of the polypeptide which reduced its ability to inhibit the RNA polymerase. The ability of syringomycin to bind to DNA also may decrease at increased pH levels and would thus account for the greater activity of the RNA polymerase in the presence of syringomycin at pH 7.4.

Whether the action of syringomycin on nucleic acid metabolism is specific for DNA-dependent RNA synthesis remains to be determined. The characterization of the interaction between syringomycin and DNA is under current investigation.

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